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**9814888.5**

10 JUL 98 E374584-7 D02884  
P01/7700 25.00 - 9814888.5

### Request for grant of a patent

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1. Your reference	P219931/LXM/RMC		
2. Patent application number (The Patent Office will fill in this part)			
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Patents ADP number (if you know it)			
If the applicant is a corporate body, give the country/state of its incorporation			
4. Title of the invention	"Peptide"		
5. Name of your agent (if you have one)	Murgitroyd & Company 373 Scotland Street GLASGOW G5 8QA		
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8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if: a) any applicant named in part 3 is not an inventor, or b) there is an inventor who is not named as an applicant, or c) any named applicant is a corporate body. See note (d))	YES		

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Description 7

Claim(s)

Abstract

*3* Drawing(s) 3

10. If you are also filing any of the following, state how many against each item.

Priority documents -

Translations of priority documents -

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*) -

Request for preliminary examination and search (*Patents Form 9/77*) -

Request for substantive examination (*Patents Form 10/77*) -

Any other documents (please specify) -

11. I/We request the grant of a patent on the basis of this application.

Signature *Murgitroyd & Company* Date  
Murgitroyd & Company 09/07/98

12. Name and daytime telephone number of person to contact in the United Kingdom Roisin McNally 0141 307 8400

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1 "Peptide"

2

3 The present invention relates to a modified analogue of  
4 the signal peptide sequence from Kaposi syndrome  
5 fibroblast growth factor (KFGF) to be used as a cell-  
6 permeant vehicle for the intracellular delivery of  
7 covalently linked anti-sense peptide nucleic acid  
8 sequences (PNAs).

9

10 PNAs have potential uses as antisense molecules for the  
11 control of gene expression. Since they are capable of  
12 binding tightly to DNA and RNA targets thus preventing  
13 DNA transcription to RNA and RNA translation to  
14 protein. These molecules thus have two potential uses  
15 of commercial importance:

16

17 1. As research reagents where scientists use  
18 antisense strategies to ablate selected genes in  
19 order to understand their function.

20

21 2. As pharmaceutical compounds for companies seeking  
22 to develop nucleic acid-based therapies.

23

24 Conventional anti-sense oligonucleotide in vivo delivery  
25 is highly inefficient, even if long-lasting, less polar

1 The invention provides modified peptide sequence I as  
2 detailed herein.

3

4 The invention also provides peptide sequences II and  
5 III as detailed herein.

6

7 The invention provides use of a peptide as defined  
8 herein together with lysine residues for multiple  
9 presentation of peptide nucleic acids.

10

11 The invention further provides use of peptides as  
12 defined herein together with lysine residues in the  
13 simultaneous presentation of different peptides nucleic  
14 acids.

15

16 The present invention combines the two above  
17 technologies to use CPPI to deliver PNAs to in vivo  
18 targets.

19

#### 20 Example

21

22 In order to determine the best delivery system, a  
23 comparison of the ability of three different cell  
24 permeant peptides to accumulate in whole cells was  
25 undertaken. The three peptides (Table 1) were labelled  
26 with carboxyfluorescein and the amount accumulated  
27 intracellularly was assayed after exposure of cells to  
28 50 $\mu$ M; peptide II = 0.4 $\mu$ M; peptide III = -0.4 $\mu$ M.

29

30 Table 1

31

32 I CFI A A V A L L P A V L L A L L A P K K K

33

34 II CFI R F A R K G A L R Q K N V H E V K N

35

36 III CFI R P R P Q Q F O G L M

37

r

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1 Key Peptide I : modified kFGF signal sequence  
2 Peptide II : PKC pseudosubstrate sequence  
3 Peptide III : modified substance P  
4 CFI : Carboxyfluorescein  
5 Or : Ornithine  
6 Boldface : Modifications to original sequence  
7

8 Peptide I was modified to contain three lysines C-  
9 terminal of the hydrophobic signal sequence. This  
10 peptide, therefore, can accommodate three PNAs, each  
11 bonded to a lysine epsilon amino group. This can be  
12 extended using the Multiple Antigen Presentation (MAP)  
13 technology to present eight (or more) PNA's on one  
14 peptide I sequence. A 'lysine tree' constructed in  
15 this way accommodates eight copies of the same PNA (see  
16 Fig 1A), thus increasing the effective concentration  
17 delivered by each CPPI. Alternatively a carrier can be  
18 constructed containing three (or more) different PNAs  
19 directed towards different sites on the same target  
20 mRNA (see Fig. 1B). This strategy has been termed  
21 'molecular triangulation' (Branch, A.D., 1998).  
22

23 Further to the sequences illustrated in Figures 1 and 2  
24 additional tri-lysine extension by providing three  
25 positive charges, appears to aid solubility and cell  
26 permeability to allow PNA sequences to be transported.  
27 Therefore in addition to using lysine residues to  
28 attach to PNA sequences, additional tri-lysine  
29 extension is recommended. Examples of presentation  
30 peptide using the additional try-lysine is demonstrated  
31 in Figures 3b, c, d and e and in Figure 4c and d.  
32

33 Lysine extensions comprising more or less than three  
34 lysine residues may also be useful to provide  
35 additional solubility and cell permeability.  
36

- 1 The lysine extension may be provided next to a
- 2 carboxyfluorescein reporter group to enhance its
- 3 fluorescence.

Fig. 1A - Multiple presentation of a single PNA species

Fig. 1A Multiple presentation of a single PNA species

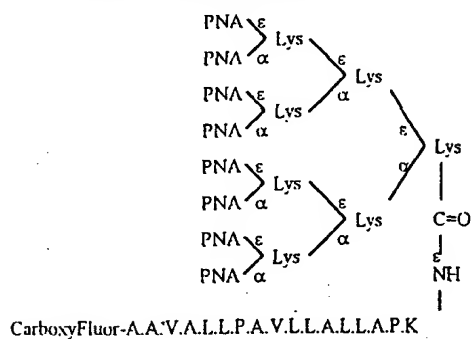
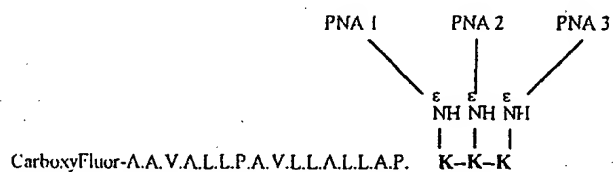


Fig. 1B - Simultaneous presentation of 3 PNAs directed to different sites on same target RNA

Fig. 1B Simultaneous presentation of 3 PNAs directed to different sites on same target RNA





## 1   References

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- 32

Fig 2. Uses of modified kFGF signal peptide in Cell Permeable Peptide Import

A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P

1 a. Native kFGF signal peptide sequence

CarboxyFluor-A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P

1 b. Signal peptide sequence with reporter group

CarboxyFluor-A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P.K.K.K

1 c. C-terminal tri-lysine extension provides 3 positive charges aiding solubility and cell permeability

CarboxyFluor-A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P-PNA SEQUENCE

PNA forms part of the linear primary amino acid sequence

CarboxyFluor-A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P-PNA SEQUENCE-K.K.K

1 d. C-terminal tri-lysine extension provides 3 positive charges aiding solubility and cell permeability

CarboxyFluor-A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P.K.K.K-PNA SEQUENCE

CarboxyFluor-K.K.K-~~A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P~~-PNA SEQUENCE

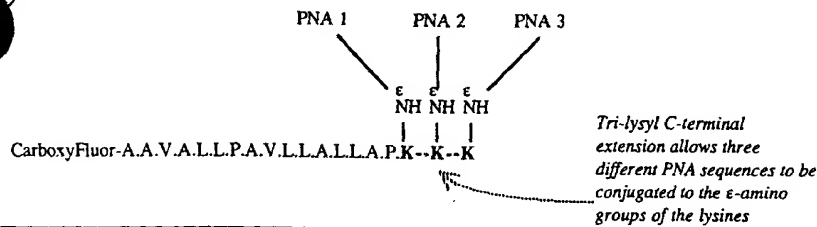
1 e. Tri-lysyl N-terminal extension provides 3 positive charges aiding solubility and cell permeability. Proximity to the carboxyfluorescein reporter group enhances its fluorescence.

CarboxyFluor-K.K.K-PNA SEQUENCE-A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P

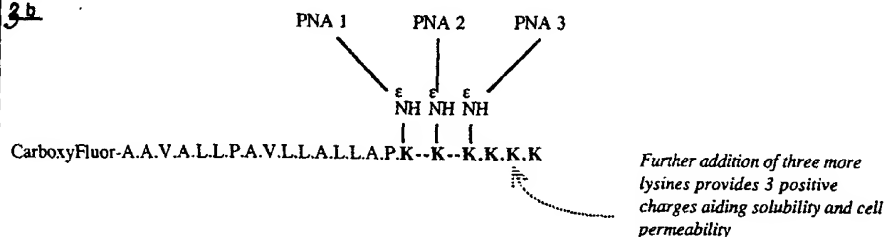
The "cargo" to be delivered intracellularly is represented as a Peptide Nucleic Acid (PNA) in Figures 1, 2 & 3. However, the various configurations of CPPI described in this patent could also be used to carry peptide sequences or oligonucleotide sequences (either native sequences or modified sequences, such as phosphothiorates).

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2a

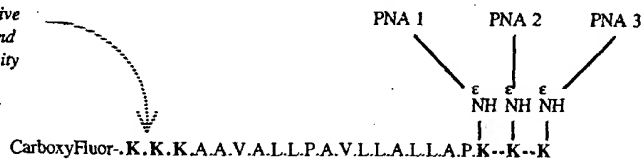


2b



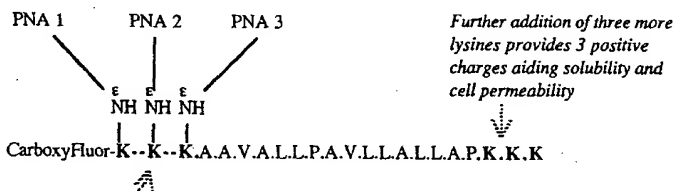
3c

Tri-lysyl N-terminal extension provides 3 positive charges aiding solubility and cell permeability. Proximity to the carboxyfluorescein reporter group enhances its fluorescence.



3d

N-terminal tri-lysyl extension added to kFGF signal peptide sequence allows three different PNA sequences to be conjugated to the ε-amino groups of the lysines



3e

Tri-lysyl N-terminal extension provides 3 positive charges aiding solubility and cell permeability. Proximity to the carboxyfluorescein reporter group enhances its fluorescence.

